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Insecticidal activities and active components of the alcohol extract from green peel of *Juglans mandshurica*

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Abstract: The extract of green peel of *Juglans mandshurica* Maxim was extracted by common method for studying its insecticidal activities and analyzing the active components. Results showed that the alcohol extract and the chloroform part of extract (separated with chloroform from alcohol extract) form green peel of *J. mandshurica* have insecticidal activities in contact toxicity and stomach toxicity against larvae of *Lymantria dispar* L.. After application of the extracts for five days, the corrected mortality of larvae of *Lymantria dispar* for both extracts was more than 50% in contact toxicity and stomach toxicity at the concentration of ≥ 5 g·L^{-1}. The insecticidal activity for both alcohol extract and chloroform part of extract is more effect in contact toxicity than in stomach toxicity, but no significant difference in the insecticidal activities was found between alcohol extract and chloroform part of extract. The active components in the chloroform part of extract from green peel of *J. mandshurica* were analyzed by GC-MS. The analyzed results showed that the active components in the chloroform part of extract are: (1) juglone (5-hydroxy-1,4- naphthaoquinone), the relative content 27.11%, (2) 1,5-Naphthalenediol, the relative content 9.52%, (3) 7-Methoxy-1-tetralone, the relative content 6.81%, (4) Benzofuran, 2,3-dihydro-, the relative content 6.76%, (5) 4-Hydroxy-2-methoxycinnamaldehyde, the relative content 3.99%, (6) 2-Methoxy-4-vinylphenol, the relative content 3.05%.

Keywords: Green peel, *Juglans mandshurica* ; Insecticidal activities; Component analysis; GC-MS; Juglone

Introduction

Juglans mandshurica Maxim is one of the pharmaceutical hardwood in northeast and north of China (Sun 2004). The green peel of *J. mandshurica* has anti-neoplasm (Wang *et al*. 1995). The chloroform and benzene extracts of green peel of *J. mandshurica* inhibits germination, growth and formation of chlorophyll of *Raphanus sativus*, *Lactuca sativa*, *Echinochloa crus-Aalli* and *Digitaria sanguinalis* (Piao *et al*. 2006).

The isolation of oligomeric juglones is reported from the fresh bark of *J. mandshurica* (Hirakawa *et al*. 1986). Four known flavonoids and two galloyl glucoses were isolated from the stem-bark of *J. mandshurica* (Min *et al*. 2003). Four new diarylheptanoids, along with two known tetralones, were isolated from the roots of *J. mandshurica*, and their structures were elucidated on the basis of spectroscopic studies (Li *et al*. 2003). Six compounds (juglone *et al*.) were isolated from the leaves of *J. mandshurica* (Wu *et al*. 1994).

Juglone is main toxic substance, and there are lower toxicities for other active substances in *J. mandshurica* (Xu *et al*. 1990). The volatile oil extracted from the stem-bark of *J. mandshurica* by steam distillation was analyzed by GC/MS, and 39 compounds in it have been identified (Wang *et al*. 2005). The extractions of total flavonoid and juglone from leaves, bark and green peel of *J. mandshurica* trees were carried out by three extracting methods as common method, reflux method and ultrasonic method (Sun *et al*. 2006).

It is estimated that there are more than 1000 kinds of insecticidal plants in China..In this study, we observed the insecticidal activities of the alcohol extract and chloroform part of extract (separated with chloroform from alcohol extract) extracted from green peel of *J. mandshurica* and analyze the active components of chloroform part of extract by GC-MS, with a purpose of providing the theoretical proof for exploitation and utilization of insecticidal plants and for simulating the synthesizing of botanical pesticides

Materials and methods

Materials

The green peel samples of *J. Mandshurica* were collected from Mao'ershan Experiment Station of Northeast Forestry University. The 2nd ~ 3rd instar larvae of *Lymantria dispar* L. were obtained from insect breeding laboratory in Northeast Forestry University. *Lymantria dispar* L. is one of harmful forest insects in northeast and north of China (Shi 2003).

Test methods of insecticidal activities

The extract of green peel of *Juglans mandshurica* Maxim was

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extracted with 95% alcohol by common method. The alcohol extract was concentrated with vacuum distillation, and separated with petroleum ether, chloroform, and ethyl acetate according to the polar difference.

The insecticidal activities of alcohol extract and chloroform part of extract (separated with chloroform from alcohol extract) were tested on the 2^{nd}–3^{rd} instar larvae of *Lymantria dispar* for five days. Five concentrations of extract samples were designed as 50, 10, 5, 1 and 0.5 g·L^{-1}. Thirty 2^{nd}–3^{rd} instar larvae of *Lymantria dispar* were used for each test concentration and three repeats were placed for each concentration. The control container with solvent but without the extract was used in test.

The larvae were put into the extract solution for 5 s for testing the insecticidal activities of contact toxicity. The insects were fed with fresh leaves of poplar every day. Fresh leaves of poplar were put into the extract solution for 5 s, then, they were used to feed the larvae at the same hungry degree for testing insecticidal activities in stomach toxicity.

Analysis methods of active components

The active components and relative contents in the chloroform part of extract on green peel of *J. mandshurica* were analyzed by GC-MS. Agilent 6890N-5973 inert made in USA was used. Condition of GC were as follows: DB-17MS capillary column, length 30m, radius 0.25mm, film 0.25μm, temperature 260 ºC in gaseous room, with helium gas, flow 1 mL·min^{-1}, inject amount 1μL, original temperature 50 ºC, elevated 5 ºC·min^{-1} to 250 ºC, hold 5 min, GC-MS maximum temperature 290 ºC. Condition of MS were as follows: EI electron energy of ionic resource 70 eV，temperature of ionic resource 230 ºC, Scan range15-260 amu。

Results and analysis

Insecticidal activities of alcohol extract and chloroform part of extract

Insecticidal activities of alcohol extract and chloroform part of extract of green peel of *J. mandshurica* against *Lymantria dispar* after feeding with extracts for five days were shown in Table 1.

Table 1. Insecticidal activities of extracts from green peel of *Juglans mandshurica* Maxim on larvae of ***Lymantria dispar* L.**

Concentration (g·L^{-1})	Corrected mortality *L* (%)			
	Alcohol extract		Chloroform part of extract	
	Contact poison	Stomach poison	Contact poison	Stomach poison
Control	0	0	0	0
0.5	48.3	40.0	43.3	35.0
1	45.0	48.3	46.7	45.0
5	53.3	55.0	56.7	50.0
10	70.0	63.3	73.3	65.0
50	95.0	80.0	98.3	85.0

Both alcohol extract and chloroform part of extract exhibit insecticidal activities in contact toxicity and stomach toxicity against *Lymantria dispar*. The corrected mortality is more than 50% when the concentration of the extract is above 5 g·L^{-1} and the insecticidal time is above five days for both extracts. The insecticidal activity in contact toxicity is more effect than stomach toxicity for both alcohol extract and chloroform part of extract, but no significant difference in the insecticidal activities was observed between alcohol extract and chloroform part of extract.

Analysis of active components of chloroform part of extract

The analysis of chemical components and relative contents is accomplished according to the peak area by Nist02 GC-MS information and data base. The total ion current chromatogram of chloroform part of extract by GC-MS was as shown in Fig. 1.

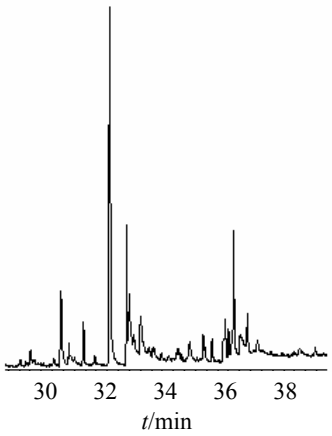

Fig. 1 Total ion current chromatogram of chloroform part of extract

The chemical components and relative contents in chloroform part of extract of green peel of *J. mandshurica* by GC-MS was analyzed as shown in Table 2.

The main chemical components in chloroform part of extract from the Table 2 are: juglone (5-hydroxy-1,4- naphthaoquinone), the relative content 27.11%, and 1,5-Naphthalenediol, the relative content 9.52%, and phenols and esters by GC-MS.

Because esters have no insecticidal activities (Tan *et al*. 2002), the activity components in chloroform part of extract might be: juglone (5-hydroxy-1,4- naphthaoquinone), the relative content 27.11%, 1,5-Naphthalenediol, the relative content 9.52%, 7-Methoxy-1-tetralone, the relative content 6.81%, Benzofuran, 2,3-dihydro-, the relative content 6.76%, 4-Hydroxy-2-methoxycinnamaldehyde, the relative content 3.99%, and 2-Methoxy-4-vinylphenol, the relative content 3.05%.

Conclusions

The study results showed that the alcohol extract and the chloroform part of extract (separated with chloroform from alcohol extract) from green peel of *J. mandshurica* have insecticidal activities in contact toxicity and stomach toxicity against larvae of *Lymantria dispar.* The corrected mortality exceeds 50% when the concentration of the extract is equal to or higher 5 g·L^{-1} and the insecticidal time is more than five days for both extracts. The insecticidal activity in contact toxicity is more effect than stomach toxicity for both alcohol extract and chloroform part of extract, but there is no significant difference in the insecticidal activities between alcohol extract and chloroform part of extract.

The analyzed results showed that the active components in chloroform part of extract on green peel of *J. mandshurica* might be: (1) juglone (5-hydroxy-1,4- naphthaoquinone), the relative

content 27.11%, (2) 1,5-Naphthalenediol, the relative content 9.52%, (3) 7-Methoxy-1-tetralone, the relative content 6.81%, (4) Benzofuran, 2,3-dihydro-, the relative content 6.76%, (5) 4-Hydroxy-2-methoxycinnamaldehyde, the relative content 3.99%, (6) 2-Methoxy-4-vinylphenol, the relative content 3.05% by GC-MS.

Table 2. Chemical components and relative contents in chloroform extract by GC-MS

No.	Compounds	Retention time (min)	Molecular mass	Formula	Relative Content (%)	Similarity (%)
1	2-Pentanone, 4-hydroxy-4-methyl-	29.60	116	$C_6H_{12}O_2$	1.01	70
2	Benzofuran, 2,3-dihydro	30.71	120	C_8H_8O	6.76	80
3	2-Methoxy-4-vinylphenol	31.53	150	$C_9H_{10}O_2$	3.05	95
4	1,4-Naphthalenedione, 5-hydroxy-	32.48	174	$C_{10}H_6O_3$	27.11	96
5	7-Methoxy-1-tetralone	33.10	176	$C_{11}H_{12}O_2$	6.81	82
6	Hexadecanoic acid, ethyl ester	33.19	284	$C_{18}H_{36}O_2$	8.72	99
7	E-11-Hexadecenoic acid, ethyl ester	33.36	282	$C_{18}H_{34}O_2$	4.26	97
8	1,5-Naphthalenediol	33.61	160	$C_{10}H_8O_2$	9.52	96
9	Phytol	35.38	296	$C_{20}H_{40}O$	2.93	87
10	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	35.88	180	$C_{10}H_{12}O_3$	2.37	93
11	4-Hydroxy-2-methoxycinnamaldehyde	36.68	178	$C_{10}H_{10}O_3$	3.99	94
12	Ethyl Oleate	36.80	310	$C_{20}H_{38}O_2$	5.50	99
13	9,12-Octadecadienoic acid, ethyl ester	36.98	308	$C_{20}H_{36}O_2$	8.74	99
14	9,12-Octadecadienoic acid (Z,Z)-	37.20	280	$C_{18}H_{32}O_2$	6.24	97
15	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	37.48	306	$C_{20}H_{34}O_2$	3.78	99

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